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(54) Title: BACTERIA TRANSFORMATION KIT

(57) Abstract: Transformation is an important step in DNA cloning, which is one of the most important component of recombinant DNA technology and many other areas of molecular biology. Bacteria transformation kit provides an easy way to transform bacteria by plasmids in a short period of time by the formulation prepared with lipid-plasmid complexes and the method mentioned below.

BACTERIA TRANSFORMATION KIT

DESCRIPTION OF THE INVENTION

Technical Area Related with Invention: This invention is related with a product or a method to be used in bacterial transformation for molecular cloning and other purposes. The area of method and product is related with microbiology, recombinant DNA technology and pharmaceutical biotechnology, but the method and the product have no connection with the methods being used in the diagnosis and treatment of diseases.

Transformation of foreign genetic materials into cells by lipids has become a common technique for mammalian cell systems. But transformation efficiency of these carriers for bacteria and yeast cells haven't been investigated yet.

Previous Techniques: Electroporation and methods requiring the preparation of competent bacteria, are widely used for bacterial transformation. Electroporation requires expensive equipment and the optimization of the method is difficult. Results obtained from one experiment is not usually reproducible.

In electroporation, DNA, intended to be put into the cell, is placed in a cuvette together with host cell suspension, between two electrodes and an electrical current is applied. Usually 250 μF current is applied every 4 seconds. Some parts of cytoplasmic membrane is subjected to a reversible breakdown when a strong current is applied for a brief period of time, and DNA molecules enter the cells. Method is rapid and applicable for every cell type but it is expensive and if the application period of current is not optimized properly, host cells die and lowers the number of surviving bacteria.

Methods requiring the preparation of competent bacteria, are difficult and time consuming. Due to this reason, most of the investigators are purchasing marketed ready-made competent bacteria stored and shipped as frozen; but stability and finance problems are encountered here. Methods used for preparing competent bacteria, need at least one night incubation. Method is as follows:

1. Inoculate bacteria in a 50 ml tube containing 15 ml Luria-Bertani (LB) broth growth medium.
2. Incubate overnigh at 37°C preferentially by shaking.
3. Inoculate 50 μl of the overnight culture into 15 ml of fresh LB broth.
4. Incubate for 2 hours at 37°C. After incubation, centrifuge the tube to obtain bacterial pellet.
5. Discard the supernatant, resuspend the pellet in 1.5 ml cold $CaCl_2$ solution and incubate on ice for one hour.

6. Centrifuge to pellet the cells and discard the supernatant.
7. Resuspend the pellet in 1.5 ml cold $CaCl_2$ solution again and incubate overnight at 4°C.
8. After overnight incubation add your plasmid to 200µl of competent bacteria and place the tube 35 minutes on ice, 1.5 minutes on 42°C water bath and again 10 minutes on ice.
9. Add 1ml of LB broth to the transformed bacteria and incubate at 37°C for one hour.
10. Inoculate bacteria to the appropriate selective medium and continue incubation at 37°C until the colonies become visible.

As can be seen from the descriptions above, classical methods either require several days for preparation or expensive equipment like electroporators. They are either long and cumbersome or harmful to bacteria due to electrical current used. Bacterial transformation kit has the advantages of providing materials and a practical method to transform bacteria by plasmids, within 45 minutes.

Objectives of the Invention Subject

1. To develop a very economical rapid method instead of a conventional procedure which requires expensive equipment.
2. To shorten a 2-3 days long conventional experimental procedure to a very short period of time such as 15-60 minutes.
3. To speed up biotechnological procedures by speeding transformation, a main step for molecular cloning and provide a time-based profit.
4. To simplify multistep and difficult procedures into a practical, rapid method.
5. To develop a highly reproducible and efficient method of transformation instead of low reproducible conventional methods.
6. To develop a product for providing the materials needed for the easy transformation protocol.

Explanation of the Invention Subject

Bacteria transformation kit is a product that provides the materials and the method needed for rapid and easy transformation of bacteria, which is widely used as a main step in recombinant DNA technology and many other research procedures related to molecular biology. By using currently available conventional methods, procedures can either be

completed in a few days or expensive equipment are required for the rapid ones. Furthermore, reproducability and yield can not be reached every time as desired. Bacteria transformation kit provides an easy and rapid transformation method and materials without necessitating specific apparatus or detailed procedures.

Fundamental principle and innovation of the method is based on use of a cationic lipid-surfactant system to carry the plasmid DNA into bacteria and a specific washing procedure using bovine serum albumin (BSA). These type of systems are started to be used widely in mammalian cells but effectiveness of the systems on bacteria or yeast cells have not been investigated yet.

Bacteria transformation kit can deliver the plasmids into bacteria in a very short time period without giving harmful effect and bacterial transformation can be achieved with high yield and reproducibility.

Bacteria transformation kit is composed of 3 solutions which are A, B and M solutions. Solution A contains 50 mM $CaCl_2$, solution B contains 5% m/v BSA (Bovine Serum Albumine) and solution M contains 1.25 % v/v (N(1-(2,3-dioleyloxy)propyl)-N,N,N, trimethyl ammonium bromide) / (dioleoylphosphatidylethanolamine) (DOTAP/DOPE) and 0.05 % m/v zwitterionic surfactant with 8 carbons. Procedure of bacteria transformation by using bacteria transformation kit is as follows:

1. Prepare a culture of bacteria in a 50 ml tube containing 15 ml Luria-Bertani (LB) broth or another appropriate growth media. Incubate overnigh at 37°C preferentially by shaking.
2. Inoculate 300 µl of the overnight culture into 15 ml of fresh LB broth. After incubation at 37°C for 2 hours centrifuge culture media to obtain bacterial pellet.
3. Resuspend the pellet in 1 ml cold solution A (50 mM $CaCl_2$) and vortex to suspend. Centrifuge and discard the supernatant.
4. Resuspend the pellet in 200 µl solution A (50 mM $CaCl_2$). Add 0.1-10 µg plasmid with or without solution M after mixing at 1/5-1/2 v/v ratio at a volume of 1-20 µl. Place the tube consequently 15 minutes on ice, 1.5 minutes on 42°C water bath, and 3 minutes on ice.
5. Centrifuge mixture to obtain bacterial pellet and discard the supernatant. Add 500 µl solution B (5 % m/v BSA), mix by vortexing, centrifuge to obtain bacterial pellet and discard the supernatant.
6. Resuspend the pellet in 200-300 µl LB broth by vortexing.

7. By using one plate for each 50-100 µl of transformed bacterial suspension, inoculate bacteria to selective medium.

Number of transformant bacteria is incread after washing with BSA. This result is probably related with removal of the toxic products from media, which are harmful for bacteria. Method can be carried out either with fresh or overnight grown bacteria, but higher transformation efficiency is obtained with fresh bacteria. Transformation efficiency is increased further by incubating bacteria with lipid complexes.

CLAIMS

1. Use of 5 % m/v BSA as a washing solution to increase bacterial transformation and survival.
2. Use of lipids like 1.25 % v/v (N(1-(2,3-dioleyloxy)propyl)-N,N,N, trimethyl ammonium bromide) / (dioleoylphosphatidylethanolamine) (DOTAP/DOPE) mixture and surfactants like 0.05 % m/v zwitterionic surfactant with 8 carbons for increasing bacterial transformation efficiency.
3. A transformation kit containing 50 mM $CaCl_2$ and 5 % m/v BSA solutions for transforming easy and rapid transformation of bacteria.
4. A seperate kit containing 50 mM $CaCl_2$ and 5 % m/v BSA and 1.25 % v/v (N(1-(2,3-dioleyloxy)propyl)-N,N,N, trimethyl ammonium bromide) / (dioleoylphosphatidyl-ethanolamine) (DOTAP/DOPE) and 0.05 % m/v zwitterionic surfactant with 8 carbons together with the solutions mentioned in the 3rd claim.
5. Easy and rapid transformation method provided with the kits mentioned in the 3rd and 4th claims.
6. Use of the kits mentioned in claims 3 and 4 for the transformation of foreign genetic material to yeasts.

INTERNATIONAL SEARCH REPORT

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CLASSIFICATION OF SUBJECT MATTER

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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC[7]: C12N 1/21, 15/00, 15/63

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, CAS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5703055 A (FELGNER et al.) 30 December 1997 (30.12.97) column 24, line 61 - column 26, line 67, column 29, lines 11-44.	2
X	WO 98/07408 A1 (THE GOVERNMENT OF THE UNITED STATES OF AMERICA AS REPRESENTED BY THE SECRETARY DEPARTMENT OF HEALTH AND HUMAN SERVICES) 26 February 1998 (26.02.98). page 2, 2nd paragraph; page 7, line 6 - page 9, line 16; example 4.	2

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

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Information on patent family members

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